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13. ABSTRACT *(Maximum 200 words)*

This annual report documents our progress in the initial phase of our study of the influence of diet on CCAAT/Enhancer binding proteins (C/EBPs) in mammary tissue. Our goal is to determine if chronic high fat or excess caloric intake alters C/EBP subcellular localization or DNA binding activity and the relationship between this diet-induced effect on mammary tumorigenesis. Diet-induced alterations in C/EBPs could provide a biochemical link between diet and mammary tumor promotion.

Female MMTV/c-neu transgenic mice and nontransgenic controls are being fed 5% or 20% fat (w/w) diets ad libitum or at 20% caloric restriction (four diets total). The data in this report demonstrate the influence of the diets on body weights after 2 months of feeding. The results indicate that there is no difference in body weights between the ad libitum fed groups (Diet 1 vs 2). The body weights of both ad libitum fed groups are statistically greater than the restricted fed groups (Diets 3 & 4). At this point in the study, the body weights of group 4 are greater than group 3. Mammary tumors develop in these mice at about 6 months of age, therefore, we have not detected any tumors at this point in the study. The in vivo feeding and tumor incidence phase of the study will be completed by November 1995.

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/ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

James L. Ouellette 8/11/95

PI - Signature Date

4. TABLE OF CONTENTS

5. INTRODUCTION

A. Nature of the problem.

The nature of the problem under investigation in this proposal is the role of diet as a breast cancer risk factor. Breast cancer is the number one form of cancer and the second leading cause of cancer deaths among US women (15). Of the known breast cancer risk factors (age, sex, diet, family and reproductive history) only diet could be realistically modified on a population-wide basis to reduce breast cancer incidence (16). National health agencies recommend a general reduction in fat intake to reduce breast cancer risk (16,17,19). Clinical trials with very low fat diets are in progress with high risk women and breast cancer patients (18,19). In addition, the historic Women's Health Initiative will test low vs high fat diets in a 10 year study enrolling 70,000 women (17-19).

Despite these efforts the "diet/breast cancer" hypothesis remains controversial (17). What is lacking is a precise definition of the link between diet and breast cancer. Without a biochemical mechanism, dietary recommendations will continue to be relatively nonspecific (i.e., decrease tat intake). In addition, our limited understanding prevents any determination regarding which women would benefit most from strict adherence to dietary modification, and when these modification should be implemented to maximize their preventative effects.

Our hypothesis is that diet acts as a mammary tumor promoter by inducing alterations in CCAAT/Enhancer binding proteins (C/EBPs). C/EBPs are a unique family of transcription factors implicated in the control of genes involved in energy metabolism, cell growth and differentiation (1-10). Aberrant expression and function of transcription factors plays an important role in tumorigenesis (20,21).

B. The background of previous work.

Our lab is among the first to investigate transcription factors as the biochemical link between diet and mammary tumor promotion. We identified a unique pattern of C/EBPs in mammary tissue and found that differentiation and growth influence C/EBP isoform subcellular localization (11).

This proposal will extend these preliminary findings and investigate the influence of diet on C/EBP subcellular localization and DNA binding activity in mammary tissue. This approach clearly constitutes a new perspective on the long standing issue of diet as a breast cancer risk factor. The results will provide new information regarding mechanisms underlying diet and mammary tumor promotion.

With the incidence of breast cancer rising in the US it is imperative that we gain a better understanding of dict as a controllable breast cancer risk factor. At the basic level, it is equally imperative that we investigate cellular factors, such as C/EBPs, that are implicated in mammary cell growth control and differentiation and may link diet and mammary tumor promotion.

Current public health policy regarding breast cancer is limited to encouraging early detection of existing disease. A better understanding of the biochemical link between diet and breast cancer will provide a basis for more specific and more effective preventative public health recommendations to reduce breast cancer incidence before the end of the 1990's.

C. The purpose of the present work

The specific purpose of this proposal is to investigate the influence of diet on CCAAT/ Enhancer binding proteins (C/EBPs) in mammary tissue. C/EBPs are a family of DNA binding proteins implicated in the control of energy metabolism, growth and differentiation (1-10). Mammary tissue expresses a unique pattern of C/EBP isoforms that translocate between the nucleus and cytoplasm in a growth and differentiation-dependent manner (11, Figures 1-3). Our goal is to determine if chronic high fat or excess caloric intake alters C/EBP subcellular localization or DNA binding activity.

Diet-induced alterations in C/EBPs could provide a biochemical link between diet and mammary tumor promotion. A better understanding of the link between diet and breast should translate into more effective public health recommendations to modify dietary practices and reduce breast cancer risk.

D. The methods of the approach.

Female MMTV/c-neu transgenic mice and nontransgenic controls are fed 5 % or 20% fat diets ad libitum or at 20% caloric restriction. The influence of the experimental diets on mammary gland and mammary tumor C/EBP isoform subcellular localization (Western blot) or DNA binding activity (Mobility shift assay) will be assessed. MMTV/c-neu mice overexpress the cneu protooncogene and develop invasive ductal adenocarcinomas (IDA) that undergo metastasis (12). These unique characteristics make the results from experiments with MMTV/c-neu mice highly relevant to human breast cancer as cneu overexpression, development of IDAs, and metastasis are common in human breast cancer (12-14).

6. BODY **(Includes responses to specific comments by reviewers to annual report).**

SUMMARY REVIEW:

1. Results show no statistical differences in body weights...

Body weight data for the full study is presented in this revised report (Figure 1). The results indicate significant statistical differences in body weights between the ad libitum fed groups (Diet 1 vs 2) and the restricted fed groups (Diets 3 & 4). The body weights of both ad libitum fed groups are statistically greater than the restricted fed groups (Diets 3 & 4). The body weights of the high fat groups (Diets 2 and 4) are greater than the low fat groups (Diet 1 and 3).

FORMAT/EDITORIAL ISSUES:

1. References 20 and 21 are missing:

20. Cox PM and CR Goding. Transcription and cancer. Brit. J. Cancer, 63:651-662, 1991.

21. Cleary ML. Oncogenic conversion of transcription factors by chromosomal translocations. Cell, 66:619-622, 1991.

CONTRACTUAL ISSUES:

Discrepancies in the statement of work..

1. Weight data for 2 months vs 200 days

As discussed above body weight data for the full study is presented in Figure 1. The results indicate significant statistical differences in body weights between the ad libitum fed groups (Diet 1 vs 2) and the restricted fed groups (Diets 3 & 4). The body weights of both ad libitum fed groups are statistically greater than the restricted fed groups (Diets 3 & 4). The body weights of the high fat groups (Diets 2 and 4) are greater than the low fat groups (Diet 1 and 3).

Although not addressed in the reviewer's comment the mammary tumor incidence data is complete. Mammary tumor incidence was higher in the ad libitum vs the restricted fed groups. The highest incidence of mammary tumors was in the 20% corn oil ad libitum fed group. Statistical analysis of the mammary tumor data is in progress.

2. Western blots should have been performed...

A representative Western blot is presented in Figure 2 (A-C). The Western blot analysis is in progress.

3. Some reports on DNA binding should have been reported...

A representative band shift assay is presented in Figure 3. The band shift analysis is in progress.

SPECIFIC DISCREPANCIES AND RECOMMENDATIONS:

..explanation of the change in the time of animal observation (from 200 days to 60 days) and the reasons for the lag in the schedule..

The study was somewhat delayed due to problems with the supply of mice required for the study. As discussed above we encountered unanticipated difficulties in obtaining both transgenic mice and the nontransgenic FVB (control) females. FVB females are in great demand because the eggs derived this strain are larger and survive the microinjection process better than other mouse strains.

Thank you for the opportunity to reply to your reviewers comments. If you require additional information please do not hesitate to contact me by mail at my academic department or by phone (614-292-4261 or FAX 614-292-6473).

The protocol was designed to test the effects of ad libitum feeding and fat content of the diet on body weights, mammary tumors and mammary tumor and mammary gland C/EBP isoform content. This report summarizes the data that has been completed describing the effects of the diets on body weights after study completion.

The data demonstrate body weight differences between growing female mice provided nutritionally complete diets ad libitum vs restricted fed. The body weights of ad libitum fed female MMTV/c-neu mice are statistically greater than the restricted fed groups. Restricted fed groups (groups 3 & 4) are fed 80% of the energy intake of their ad libitum paired group (groups 1 & 2).

These results are consistent with one of the goals of the study which is to assess the influence of energy restriction, regardless of fat content, on body weight and mammary tumorigenesis.

7. CONCLUSIONS

a. Summary of implications of completed data.

(1) Body weights of growing female MMTV/c-neu and FVB controls fed low or high fat diets ad libitum are statistically different than mice fed the same diets but restricted in total caloric intake.

(2) The body weights of the restricted fed group fed the high fat [20% corn oil (w/w)] are statistically greater than restricted fed mice fed a low fat [5 % corn oil (w/w)].

b. Recommended changes.

As discussed in my response of November 18, 1995 and summarized above the study was unavoidably delayed due to the short supply of female FVB mice, which are commonly used in the development of transgenic mice from commercial sources. Despite this delay the study is on schedule.

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of the gene encoding CCAAT/enhancer binding protein. Genes & Dev 3:1146-1156, 1989.

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21. Cleary ML. Oncogenic conversion of transcription factors by chromosomal translocations. Cell, 66:619-622, 1991.

Figure 1.

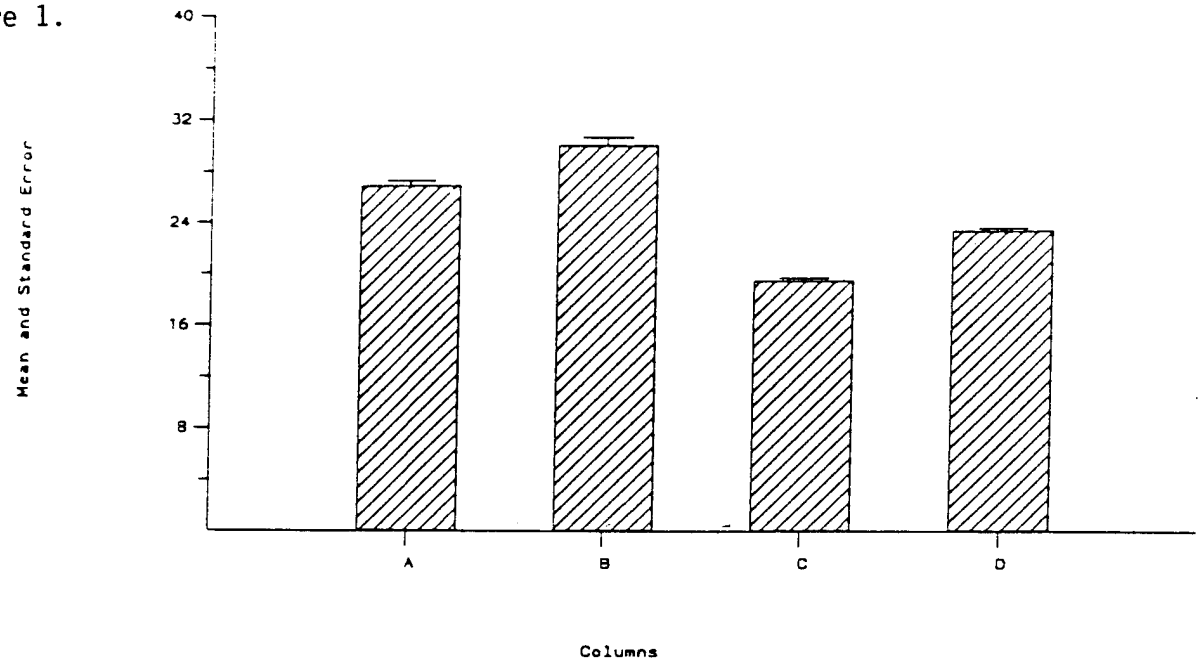
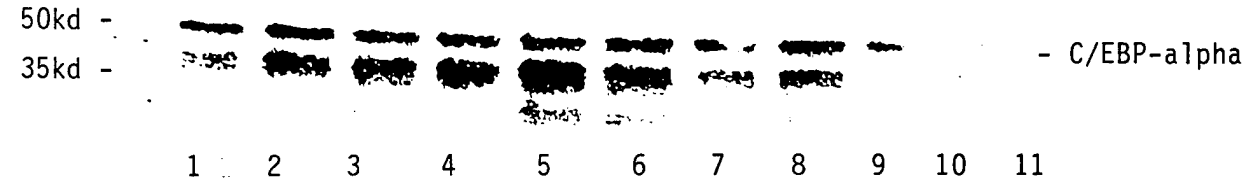


Figure 1. Final body weights of experimental mice fed 4 diets. Column A (diet 1, 5% corn oil, (CO) adlibitum); Column B (diet 2, 20% CO, ad libitum); Column C (diet 3, 5% CO, restricted fed); Column D (diet 4, 20% CO, restricted fed). Mean body weights: diet 1: 26.86 +/- 0.42; diet 2, 30.03 +/- 0.67; diet 3: 19.46 +/- 0.26; diet 4: 23.41 +/- 0.28 (mean +/- SEM)

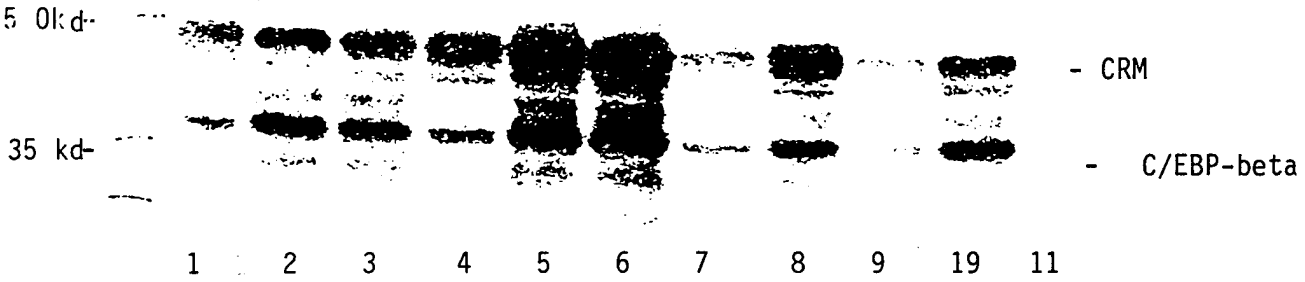
Tukey-Kramer Multiple Comparisons Test
If the value of q is greater than 3.669 then the P value is less than 0.05.

Comparison	Mean Difference	q	P value
Diet 1 vs Diet 2	-3.160	7.006	*** P<0.001
Diet 1 vs Diet 3	7.409	15.907	*** P<0.001
Diet 1 vs Diet 4	3.459	7.393	*** P<0.001
Diet 2 vs Diet 3	10.569	23.030	*** P<0.001
Diet 2 vs Diet 4	6.619	14.356	*** P<0.001
Diet 3 vs Diet 4	-3.950	8.306	*** P<0.001

Figure 2 ; C/EBP-alpha



B. C/EBP-beta



C. C/EBP-delta

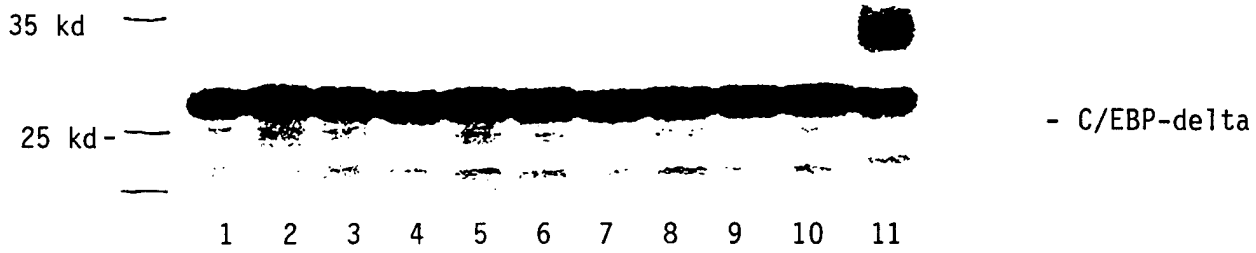


Figure 2. Western blot of C/EBP isoforms .
Mammary gland nuclear extracts from normal (control mice) fed a standard chow diet (lanes 1-11). These preliminary results indicate the detection of the C/EBP isoforms in the nucleus of mammary gland - derived nuclear extracts. CRM = cross reacting material.

Figure 3: Band shift assay

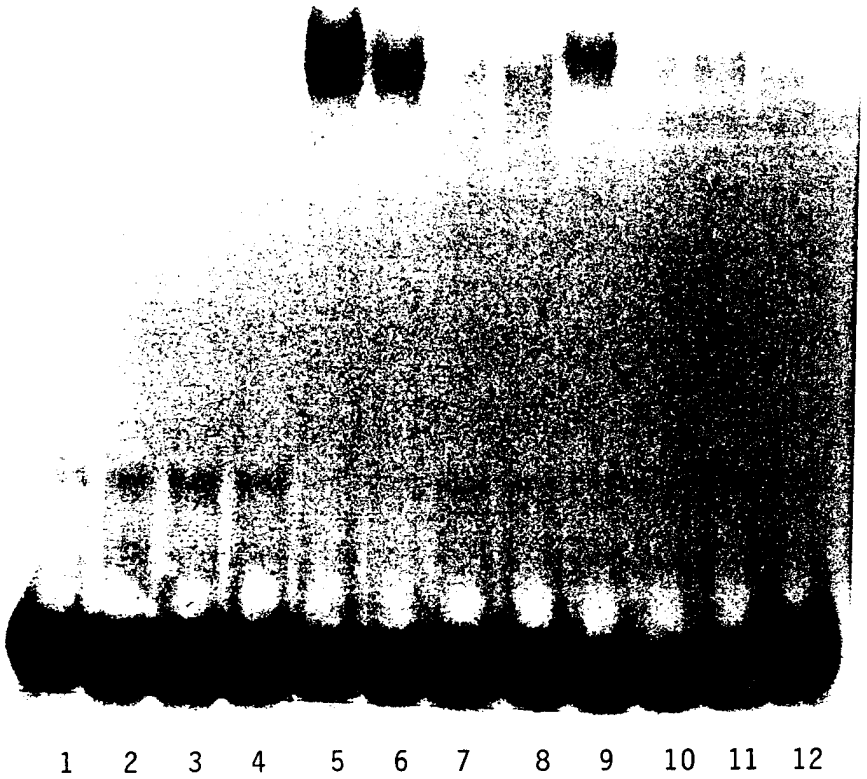


Figure 3: Band shift assay. Preliminary band shift results are presented from assays with mammary epithelial cells from pregnant mice. Probe is 32P-labelled C/EBP consensus sequence.

Lane 1: growing mammary epithelial cells

Lanes 2-4: addition of increasing amounts of anti-C/EBP-delta antisera (protein interaction data)

Lane 5: 2 days growth arrested mammary epithelial cells

Lane 6-8: protein interaction data with increasing amounts of anti C/EBP-delta antisera. Disappearance of the band shift complex indicates specific interaction of C/EBP-delta.

Lane 9: 4 days growth arrested mammary epithelial cells

Lanes 10-12: disappearance of band shift by increasing amounts of anti-C/EBP-delta antisera.

This approach will be repeated in the mammary gland and mammary tumor to confirm DNA binding by each of the C/EBP isoforms.